Pre-Ph.D. Registration Synopsis

Role of Protein Metabolism in Resistance and Susceptibility in leaves of two cabbage cultivars infected with *"Xanthomonas campestris pv campestris"*

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State of art;

Cabbage **"*Brassica oleracea cv. capitata*"** is a leafy vegetable crop which is cultivated all around the world. Cabbage (Brassica oleracea), vegetable and fodder plant of the mustard family (Brassicaceae), the various agricultural forms of which have been developed by long cultivation from the wild cabbage .This shallow-rooted,cool-season crop is cultivated for its large leafy head and is thought to have originated in western Europe. The different cultivated types of cabbage show great variation in respect of size, shape and colour of leaves as well as size, shape colour and texture of the head. This vegetable has a great economic importance because it has low calorie nutrient, moderate amount of protein, low fibres, low sodium contents, vitamin A (Beta carotene), moderte amount of vitamin B (folate), and in moderate quantity calcium mineral. The edible portions of all cabbage forms—which include kale, broccoli are low in calories and are an excellent source of vitamin C. Cabbage is highly nutritious vegetable, cabbage are rich in vitamin C, Fiber and vitamin K. They may help with heart and digestive health. The cabbage may help protect against radiation, prevent cancer and reduce heart disease risk.

This vegetable crop is oftan attack by Black rot bacterium *"**Xanthomonas campestries pv. campestris***" belong to gram –ve bacteria that causes black rot the most important disease of vegetable brassica crops worldwide. Black rot, caused by ***Xanthomonas campestris* pv. *campestris, (Xcc)***, is one of the most serious diseases of crucifers world-wide.[1,2,3] Forty-nine genotypes were evaluated for resistance under field conditions in Tanzania after artificial inoculation with *Xcc* race[4-5] *"**Xanthomonas campestries pv. campestris***" can cause disease in a large number of species of Brassicaceaea (ex.Cruciferae),including economically important vegetable brassica cropes and a number of other cruciferous crops, ornamental and weedes. typical disease symptoms include V-shaped yellow lesions and blackening of the veins.These plant pathogen are responsible for Black rot of crucifer. Black rot caused by ***Xanthomonas campestris pv. campestris (Xcc)*** is one of the most serious diseases of crucifers world-wide. To establish the efficient screening method for resistant cabbage to Xcc, different inoculation methods, inoculation positions, growth stages of seedlings, and incubation temperatures after inoculation were investigated with the seven cabbage cultivars showing different resistance degrees to the pathogen.[6,7] Black rot of Brassica spp. caused by the bacterial pathogen ***Xanthomonas campestris* pv. *campestris* (XCC),** is a seedborne disease.

Because the pathogen can easily be spread by splashing water, there is a high risk of spreading it by overhead irrigation systems during the raising of cabbage transplants.[8,9]

Problem to be investigated:

Here are some problems to be investigated in black rot of cabbage.

1. Disease index-how the disease index varies across different locations, and what factor contribute to these variation.
2. Plant-pathogen interaction –how the plant's metabolism changes during the interaction with the pathogen, and how this effects the plant's ability to defend itself.
3. Disease spread- how the disease spreads, and what factors contribute to its spread.
4. Disease control- what method can be use to control the disease and how effective these methods are.

The objectives of the Investigation & Motivation :

Objective

1. To study symptoms of the two cabbage cultivars MAHY 139, and RARE BALL F1 infected by ***Xanthomonas campestris pv. campestris.***
2. Isolation and culture preparation of the pathogen and identification of Disease through biochemical test.
3. To find out the correlation between disease, variation in protein content due to proteolytic activities with the production of amino acids and their influence on susceptibility and resistance responsible in two host cultivars.
4. Estimation of protease enzyme in diseased and healthy leaves of both varieties of "*Brassica oleraces cv. capitata"*
5. Estimation of Amion-acid in diseased and healthy leaves of both varieties.
6. Preparation of protein profile of infected and healthy leaves of both varieties of "*Brassica oleraces cv. capitata". by application SDS-PAGE (Sodium dodecyl sulphat polyacrylamide gel electrophoresis).*

Motivation

Productivity of cabbage is significantly affected by the black rot disease, which us caused by *"**Xanthomonas campestries pv. campestris***" Studying the role of protein metabolism in the resistance and susceptibility of cabbage leaves help to understand how protein degradation and synthesis influence the disease progression Ultimately it leads to the development of disease resistant cabbage varieties.

HYPOTHESIS:

Black rot of cabbage is caused by a bacterium ***Xanthomonas campestris pv. campestris.*** that blocks the plants water-conducting tissue with a mucilaginous sugar.

Cause - The bacterium ***Xanthomonas campestris pv. campestris.*** causes black rot of cabbage. It can infect many plants in the Brassica genus, including cabbage, broccoli, cauliflower and kale.

Symptoms - Include yellow, V-shaped lesions on the leaves, wilting, blackening of veins.

Spread - Black rot can be spread by infected seeds, splashing water, wind, equipment, people, insects and warm temperature.

Factors - Temperature, humidity and rainfall play a key role in the development of black rot. The bacterium prefers hot and humid conditions, and high humidity after rain, storms and dew.

Resistance - Resistance to black rot is correlated with the plant's ability to maintain enough photosynthesis to provide energy for defence. cabbage breeders can develop cultivars resistant to black rot by using molecular markers linked to resistance.

RESEARCH DESIGN AND METHODOLOGY

A). Isolation and culturing of Bacteria

1. Collection of diseased cabbage plants from different fields in and around Ranchi.
2. Isolation of bacteria from infected parts of different plants collected and re-isolation after inoculation in healthy cabbage in leaves in accordance with the Koch's postulates.
3. Culturing of bacteria in different nutrient mediums (Dye 1962)[8]

B). Identification of Bacteria:

1. **Morphological Characters**: Morphologyical characters will be studied by determining:

   a) Shape and size

   b) Gram staining by modified method of Gram

   c) Motiliy and flagella staining of bacterium as per Gray, 1926 [9]

2. **Growth characters**: Growth characters of the bacteria will be studied by

   a) color and slime formation (Dye 1962)

   b) oxygen requirement,

   c) Effect of Nacl Concentration

3. **Biochemical and physiological character** : Biochemical and physiological character will be studied by

   a) Oxidative or fermentative utilization of glucose by the pathogen,

   b) Production of acid from carbohydrate by pathogen,

   c) utilization of organic acids by the pathogen,

   d) utilization of asparagines by the pathogen,

   e) catalase activity in the presence of pathogen,

   f) Gelatin hydrolysis in presence of pathogen,

   g) pathogenic action in milk.

C). Pathological symptoms: These will be recorded as Shackleton (1962) and schaad (1983)[10] and will be authenticated By the various method:

1. The method applied for the determination of stomatal index will be the same as Cutter (1979).[11]

2. Assessment of percent disease index(PDI): Disease assessment procedure will be adopted ,same as by MC Kinney(1923)[12], Shekhawat and Chakravati (1976)[13].

3. Rate of disease increase (r) and between the intervening days(r1) will be recorded as Van Der Plank,1963[14]

D). Phytochemical studies; The phytochemical constituents of cabbage leaf during the pathogenesis of black rot bacterium "Xanthomonas campestries pv campestris" will be studied by;

1). Estimation of total protein in healthy and inoculated cabbage leaf tissue: Protein contents of two cultivars of the host in healthy and bacterium inoculated condition will be estimated by the colorimetric method introduced by Lowery et al (1951).[15]

2). Estimation of protease enzyme in healthy and diseased cabbage leaves:Proteolytic enzyme play an important role in the breakdown of leaf protein.Weidehase and Parthier(1983)[16] method, for assaying of protease activity involved the measurement of amino group liberated from protein substrate, will be followed as Mahadevan,1970.[17]

3). Estimation of total amino –acids contents in healthy and diseased leaves: change in amino-acids contents have been reported in the leaves of sudceptible bean cvs.infected with Pseudomonas ,Phaseolicola (Patel and Walker,1962,1976,Prasad,1980). [18,19,20] Estimation of amino-acids will be performed as per Moore et al 1948.[21]

4). Preparation of protein profile of healthy and infected leaves of two varieties of "*Brassica Oleraces cv. Capitata"* .

TOOLS TO BE USED IN THE COLLECTION OF DATA

Compound microscope, stage & ocular micrometer, spectrophotometer, centrifuge, camera, test tubes, conical flask, petri dishes, measuring cylinder, bottles, homogenizer, Incubator, SDS-PAGE GEL, journals and computers.

SIGNIFICANCE OF THE STUDY

The establishment of the relation between disease progress, variation in protein content due to proteolytic enzyme activities with the production of amino acid, their response on susceptibility and resistance in two host cultivars, will be helpful in detetrmining the pathogenesis and resistance of the host cultivars and would pave the way for further study in this contest.

RELEVANCES TO THE PRESENT DAY PROBLEM AND NEED TO SOCIETY

Cabbage ***'Brassica oleracea cv capitata'*** a leafy vegetable crop has a great economic importance for its low calorie nutrient, moderate amount of protein, low fat, low staturated fat, no cholesterol, high amount of carbohydrates, low fibre, low sodium content, vitamin A(Beta carotene),moderate amount of vitamin B (folate),vitamin and in moderate quantity calcium minerals but are an excellent source of vitamin C.

Cabbage cultivated in this region are commonly affected by black rot bacterium. Due to this infection there is a great economical loss. This work will be helpful in determining the nature of two host cultivars, which are cultivated frequently in this locality, in respect to resistance and susceptibility of the hosts.

CONTRIBUTION TO BE THE EXISTING KNOWLEDGE

This investigation provide valuable information about a comparative account of protein expression in both the resistant & susceptible cabbage cultivars By identifying the resistance Perkins, this study may help to reduce the use of chemical patricides & Promote the plant based immunity. Many researchers have explored different aspects of this topic, focusing on various hosts and Pathogens. However my proposed study on two host cultivars is entirely novel and remains unexplored. This research has the potential to provide new insights into host pathogen interactions, improve cultivation strategies and significantly contribute to the existing body of knowledge, there by contributing to both scientific knowledge and practical application.

FUTURE WORK

In future this work will be helpful in finding the responsible proteins and other chemical constituents contributing to resistance and susceptibility in the hosts. This will be helpful in the production of new varieties of cabbage for resistance of the disease.

REFERENCES

1. Kumar,A., 2023. Estimation of protein in two cultivars of cabbage infected with black rot bacterium *Xanthomonas camperstris pv. campestris.*Biospectra; ISSN; 0973-7057,Vol.18(2).

2. Bhushan, A. and M. Prasad, 1987. Pathochemical studies of Xanthomonas vignicola and the role of leaf amino-acids in evoking susceptible and resistant responses. Zbl. Bakt. 11,142:625-629.

3. Bhushan, A. and M. Prasad, 1987. Pathochemical studies of Xanthomonas vignicola and the role of leaf amino-acids in evoking susceptible and resistant responses. Zbl. Bakt. 11,142:625-629.

4. Massomo, S. M. S., 2004 "Evaluation of varietal resistance in cabbage against the black rot pathogen, Xanthomonas campestris pv. campestris in Tanzania." *Crop Protection* 23.4 : 315-325.

5. Massomo, S. M. S., Mabagala, R. B., Swai, I. S., Hockenhull, J., & Mortensen, C. N. 2004. Evaluation of varietal resistance in cabbage against the black rot pathogen, Xanthomonas campestris pv. campestris in Tanzania. *Crop Protection*, *23*(4), 315-325.

6. Lee, Ji Hyun, 2013 "Development of efficient screening method for resistance of cabbage cultivars to black rot disease caused by Xanthomonas campestris pv. campestris." *Research in Plant Disease* 19.2: 95-101.

7. Lee, J. H., Kim, J. C., Jang, K. S., Choi, Y. H., Ahn, K. G., & Choi, G. J. 2013. Development of efficient screening method for resistance of cabbage cultivars to black rot disease caused by Xanthomonas campestris pv. campestris. *Research in Plant Disease*, *19*(2), 95-101.

8. Krauthausen, Hermann-Josef, Norbert Laun, and Walter Wohanka. 2011 "Methods to reduce the spread of the black rot pathogen, Xanthomonas campestris pv. campestris, in brassica transplants." *Journal of Plant Diseases and Protection* 118.1: 7-16.

9. Krauthausen, Hermann-Josef, Norbert Laun, and Walter Wohanka. 2011 "Methods to reduce the spread of the black rot pathogen, Xanthomonas

campestris pv. campestris, in brassica transplants." *Journal of Plant Diseases and Protection* 118, no. 1: 7-16.

10. Dye, D.W., 1962. The inadequacy of the usual determinative test for the identification of Xanthomonas spp. N. Z. Sci. 5: 293-416.

11. Schaad, N.W. and N. Thaveechai, 1983. Black rot of crucifers in Thailand. Plant. Dis. 67:1231-1234.

12. Cutter, E.G., 1979. Plant Anatomy Part I. Cells and Tissues. Oxford and 18 H Publishing House, New Delhi, Bombay, Culcutta, second edition Pp-315.

13. Mc Kinney, H.H., 1923. Influence of soil temperature and moisture on infection of wheat seedlings by Helminthosporium sativum. J. Agricl. Res. 26: 195-217.

14. Shekhawat, P.S. and B.P.Chakravarti, 1976 Factors affecting development of bacterial leaf spot of chillies caused by Xanthomonas vesicatoria. Indian Phytopath. 29: 297-392.

15. Van Der Plank, 1963. Plant diseases epidemics and control. Academic Press, New York and London. 349 pp.

16. Lowry, O. H.; Rosebroughm, N. J.; Fan, A. L. and R. J. Randall, 1951. Protein measurement with Folin Phenol reagent. J. Bio. Chem. 193: 263-275.

17. Weidehase, R.A. and B. Parthier, 1983. Peptide hydrolase activities in seedlings and hormone-treated cotyledons of pumpkin (*cucurbita pepo*). Biomed. Biochem. Acta. 42 : 897-906.

18. Mahadevan, A.; 1970. The role of Proteolytic enzyme in disease development in: Plant disease problems. Proc. 1" Internat. Symp. on Plant pathology pp. 179-184. Indian Phytopath. Soc. New Delhi. Ed. S.P. Raichaudhury et al.

19. Patel, P.N. and J. C. Walker. 1962. Physiology of halo blight in resistant and susceptible leaves. Phytopathology, 53: 322-328.

20. Patel, P.N. and J.C. Walker, 1976. Changes in free amino acid and amide content of bean, tobacco, and ont leaves with Pseudomonas phaseolicola. P. tabaci and P. coronafaciens respectively and the leaves treated with toxin Proc.

1". Internat. Symp. on Plant Pathology: Plant disease problems (S.P. Raychaudhury et al. (Eds). New Delhi: 161-166.

21. Prasad, M., 1980. The dynamics of amino acid variation during pathogenesis and host resistance. Zbl. Bakt. II. 135: 162-184.

22. Moore, S. and W. H. Stein., 1948. Photometric method for use in the chromatography of amino acid. J. Biol. Chem. 176 367-388.

23. Gray, P.H.H., 1926. A method of staining bacterial flagella. J. Bact. 12: 273-274.

24. Bidyapati, T.H; Pandey, R. R. and M.Singh, 2006. Effect of Fungal infestation on the biochemical changes in ripe dried chilly fruits during storage. J. Indian Bot. Soc. Vol. 85, 66-70.

25. Chattopadhyay, S.B. and K. Mukherjee, 1955. Black vein diseases of cabbage in West Bengal. Sci. and Cult. 21: 107-108.

26. Dowson, W.J., 1957. Plant disease due to bacteria. 2nd Ed. At the University Press, Cambridge, Great Britain, 232 p.

27. Gupta, S.K., Raghava, R.P. and Nisha Raghava, 2004. Stomatal studies of Cowpea (Vigna unguiculata (L) Walp) cultivars in relation to Bromiconazole. J. Indian Bot. Soc. Vol. 83, 116-118

28. Hedge, JE and B.T. Hofreiter, 1962. In: Carbohydrate chemistry 17 Academic press, New york.

29. Johan, H.E., Bird, L..S. and C.W. Blackman 1960. Amino-acid in cotton varieties resistance and susceptible to Xanthomonas malvacearum. Abs. Proc. Assoc. 8" Agric. WKRS. 57: 244- 247.

30. Kabnoorkar, P.S. and S.S. Deokule, 2008. Studies on Photochemical constituents of drug "Punarnava" under storage conditions due to fungi. J. Indian bot. Soc., Vol.87(384); 256-261.

31. Kumar, A., 1994. Protein metabolism in cabbage seedling infected with Xanthomonas campestris p. campestris. Ph. D. thesis, Ranchi Univ. Ranchi. 274 pp.

32. Kumar, J., 1985. The studies of biochemistry of the host-pathogen interaction products of the tomato host and the leaf spot pathogen Xanthomonas campestris pv. vesicatoria (Doidge) Dye. Ph. D. thesis Ranchi Univ. Ranchi. 144 pp.

33. Kumar. J.. Khan, A. Z. and A. Kumar, 2000. Aberrant protein metabolism of Citrus aurantifolia leaves infected with Xanthomonas campestris pv. Citri. J. Indian bot. Soc., Vol.79; 207-208.

34. Kumar, M. and M. Prasad, 1992. Organic nitrogen metabolism of crucifer seedling in relation to their responses towards Xanthomonas campestris pv. campestris. Zentralbl. Mikrobiol. 147:92-102.

35. Miller, G.L., 1972, Anal, chem. Vol. 34, 426.

36. Samyal, S. and G. Sumbali, 2002. Post harvest Mycopatogenic loss of Tomato fruits in Jammu. J. Indian bot. Soc., Vol.81; 105-108.

37. Sinha, R.K. and M. Prasad, 1983. variations in protein content due to Proteolytic enzyme activities and their influence on suscept or resistance response in maize- Erwinia carotovora var. chrysanthemi host-pathogen system. Zbl. Mikrobiol. 138: 151- 158.

38. Singh C.T.N. and M. Prasad, 1990. Aberrant protein metabolism of tomato fruits infected by Clavibacter michiganense pv michiganense. Zbl. Mikrobiol. 145:319-322.

39. Singh C.T.N. and M. Prasad, 1991. The bacterium interaction in protein and their involvement in pathogenesis of tomato fruits by Clavibacter michiganense pv michiganense. Zbl. Mikrobiol. 146:109-116.

40. Srivastava. A.K. and M. Prasad, 1986. Fluctuations in the amino-acids constituents of bean leaves of different host responses after inoculation of Xanthomonas campestris pv. phaseoli. Zbl.